JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1981, p. 550-556
0095-1137/81/110550-07$02.00/0

Vol. 14, No. 5

Rapid Identification of Material Colonization with Group B Streptococci by Use of Fluorescent Antibody

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Received 23 March 1981/Accepted 22 May 1981

To identify women colonized with group B streptococci during parturition, we used pooled type-specific fluorescent antibody to examine vaginal swabs enriched by preincubation in selective broth medium. In preliminary experiments, group B streptococcus strain III-Bell was reliably detectable with fluorescent antibody at concentrations of $\geq 10^5$ colony-forming units per ml, achieved after 6 h of incubation of small inocula (18 to 26 colony-forming units). Of the vaginal swabs from 924 parturient women examined prospectively by both fluorescent antibody and selective bacteriology techniques, group B streptococci were isolated in 154. The sensitivity of the fluorescent antibody technique increased with increasing incubation time and ranged from 49% (3 to 6 h) to 81% (7 to 12 h) to 83% (13 to 18 h) to 93% (>18 h). Colonized mothers identified within 6 h by the fluorescent antibody technique had higher rates of vertical transmission to their newborn infants (61%) than colonized mothers whose fluorescent antibody examinations were negative within this time interval (32%; $P = 0.027$). However, because of the timing of their admissions, none of the colonized mothers of the four infants who developed early-onset group B streptococcal sepsis were identified with fluorescent antibody until after delivery. Although its sensitivity approaches selective culture methods after 6 h of incubation, fluorescent antibody examination of vaginal swabs does not appear to offer a practical approach to identifying colonized parturient women for intrapartum antibiotic prophylaxis of group B streptococcal infection.

The need for safe and effective prophylaxis of perinatal infections by group B streptococci (GBS) is well recognized (4). Both selective and unselective strategies making use of antibiotics have been advocated, and clinical trials have been aimed at colonized pregnant women before delivery (15, 21), parturient women during labor (32), and both full-term and premature newborn infants (20, 25, 27, 28). Intrapartum and postnatal prophylaxis both appear promising.

The majority of infants with early-onset sepsis (EOS) show evidence of intrauterine acquisition of infection (5, 14, 29). Attack rates are significantly increased for infants born to mothers with amnionitis or prolonged rupture of amniotic membranes or whose labor is premature (6, 29). Moreover, GBS are a common cause of puerperal as well as neonatal sepsis (9). Hence, we have elected to develop a prophylactic approach directed primarily at colonized parturient women showing obstetrical risk factors.

Prenatal vaginal cultures have been reported to be unreliable in predicting maternal colonization status at delivery (2, 31). On the other hand, conventional methods of identifying GBS carriage require at least two incubations. Thus, cultures from parturient women infrequently yield useful information until after delivery. We hypothesized that a rapid diagnostic technique for detecting GBS carriage would permit identification of colonized parturient women before delivery and define an at-risk population for intrapartum antibiotic prophylaxis.

Fluoresceinated group-specific antisera have proven useful in a number of laboratories as a means for diagnosing group A streptococcal pharyngitis (22). Since timing of antibiotic treatment is not critical for this condition, however, the technique has not found acceptance for rapid diagnosis in clinical practice (11). In their original description of pooled, type-specific fluorescent antibody (FA) used for identification of GBS, Romero and Wilkinson (26) suggested that the technique might be applicable to the rapid

diagnosis of GBS-colonized perinatal patients. In this study, we investigated the value of the FA technique for this purpose on an active obstetrical service in a large metropolitan hospital.

MATERIALS AND METHODS

Preparation of FA reagents. Type-specific antisera were raised in New Zealand white rabbits by serial intravenous inoculation with formalinized vaccines prepared from GBS types Ia (strain 090/14), Ib (strain H36B/60/2), Ic (strain A909/14), II (strain 18RS21/67/1), and III (strain D136C), according to the method of Lancefield et al. (18). Rabbits with adequate antibody response (3+ to 4+), as judged by capillary precipitin reactions with HCl extracts of the homologous organism, were exsanguinated, and the antisera were separated and stored at −70°C. Antisera for types Ib, II, and III were essentially monospecific. Antisera for types Ia and Ic showed the expected cross-reactivity based on common type-specific carbohydrate antigens.

Immunoglobulin G was separated from the rabbit antisera by the method of Garvey et al. (16) and fluoresceinated by the method of Goldman (17). In brief, the globulin-rich fractions of antisera were salted out at ⅓ saturation with ammonium sulfate, pH 7.8. Immunoglobulin G was separated by chromatography on diethylaminoethyl-cellulose (Schleicher & Schuell Co.) with 0.01 M sodium phosphate buffer, pH 7.5. Immunoglobulin G fractions were conjugated with fluorescein isothiocyanate (ICN Pharmaceuticals, Inc.) at a dye/protein ratio of 30 μg/mg. Unbound dye was removed by gel filtration on Sephadex G-25 (Pharmacia Fine Chemicals, Inc.). Purity of isolated immunoglobulin G was confirmed for each antiserum by immunoelectrophoresis before conjugation. Conjugates were concentrated to 10 mg of protein per ml, divided into portions, and stored at −70°C.

We determined the titers of the conjugates by direct staining of 10^{-2} dilutions of log-phase cultures grown in Todd-Hewitt broth. Concentrations of each conjugate of 0.5 mg of protein per ml (a dilution of 1:20) gave 4+ fluorescence for homologous organisms and ≤1+ fluorescence for heterologous organisms and thus were selected as the working dilutions. A pooled antiserum for diagnostic use was constituted with 1:5 dilutions of the four conjugates against serotypes Ia, Ib, II, and III (yielding a final concentration for each conjugate of 0.5 mg/ml). Cross-reactivity was tested with 15 clinical isolates of *Streptococcus pneumoniae*, group A streptococci, and group D streptococci.

Growth kinetics and detection of GBS and FA. The kinetics of early growth of small inocula of a representative clinical strain of GBS (III-Bell) were studied in four different media consisting of Todd-Hewitt broth (Difco Laboratories) with or without supplemental defibrinated sheep blood (5%) and antibiotics (8 μg of gentamicin sulfate per ml; reagent grade; gentamicin activity, 571 μg/ml; Schering Corp.; and 15 μg of nalidixic acid per ml; reagent grade; Sigma). Inocula were 18 to 26 colony-forming units of stationary-phase (overnight) cultures. Portions of inoculated broths were examined at hourly intervals both by the FA technique (directly) and with spread plates (at 1:50 dilution in 0.02 M phosphate-buffered saline, pH 7.4) to determine an approximate threshold concentration of GBS for detection by the FA technique.

FA technique. A selective broth medium (SBM) consisting of Todd-Hewitt broth, 5% defibrinated sheep blood, 8 μg of gentamicin sulfate per ml, and 15 μg of nalidixic acid per ml was selected as suitable for both FA studies and bacteriological confirmation. This medium is identical to that of Baker et al. (7). Clinical specimens were collected with rayon-tipped swabs (Culturette; Marion Scientific Corp.) and were inoculated directly into tubes containing 1 ml of SBM. Swabs were left in the medium during incubation at 37°C. For examination by the FA technique at various intervals after inoculation, swabs were withdrawn and spotted within the etched rings of fluorescent antibody slides (Clay-Adams). The slides were air dried and fixed by flaming followed by a 15-min acetone bath. After being rinsed in distilled water, the slides were stained with pooled GBS conjugate for 15 min and then rinsed successively in 0.02 M phosphate-buffered saline (pH 7.2) and distilled water. Cover slips were added with buffered glycerol (pH 9.5) (Meloy Laboratories), and the slides were examined at ×400 with a Zeiss transmission fluorescent microscope equipped with BG38 and BG12 excitation filters and a 500 nM barrier filter.

Bacteriological technique. Subcultures of inoculated broths to sheep blood agar plates were made at the time of each examination by the FA technique. After reincubation for 24 h, morphologically compatible colonies were picked for further characterization. GBS were identified by ability to produce augmented hemolysis with *Staphylococcus aureus* beta-hemolysin (CAMP reaction). As deemed appropriate, other coccal isolates were characterized by production of catalase and coagulase (*S. aureus* and *Staphylococcus epidermidis*) and coagglutination (streptococci of serogroups A, C, D, and G; determined by Phadebact, Pharmacia).

Evaluation of the FA technique. A pilot study of the system was carried out during July and August, 1977, with vaginal and rectal swabs from 87 prenatal clinic patients. Each swab was inoculated in SBM and examined by the FA technique and subculturing at 3 h and 24 h after collection. In this study, swabs containing coccal organisms fluorescing ≥2+ (definite fluorescence, clear-cut cell outlines, and fuzzy, nonstaining cell centers) were considered positive. FA examinations and culturing were performed independently, and the results were correlated on completion. GBS strains from swabs yielding false-negative results were reexamined by the FA technique. False-positive results were further characterized by identification of fluorescent non-GBS isolates.

The FA test was then employed in a prospective study of 924 women presenting with premature labor (≤36 weeks gestation), premature rupture of membranes (before labor onset), or intrapartum fever (≥37.6°C) between September, 1977, and April, 1979. Vaginal swabs were obtained on admission by obstetrical residents, inoculated in SBM, and incubated at 37°C in the Labor and Delivery Suite at Michael

Reese Hospital and Medical Center. Swabs from 787 women were examined by the FA technique within 18 h of collection. Each swab was reexamined after an additional 24 h of incubation. Swabs from the remaining 137 women were examined on one occasion only after more than 18 h of incubation. Broth cultures were subcultured at the time of each FA examination. In this study, we required at least 3+ fluorescence (brilliant fluorescence, clear-cut cell outlines, and sharply defined nonstaining cell centers), definite streptococcal morphology (three or more non-cell-associated chains containing 3 to 5 cocci per chain), and the presence of exfoliated vaginal epithelial cells as criteria for a positive FA result. FA examinations and culturing were carried out independently, and the results were correlated on completion. GBS isolated from either subculture were considered culture proof of GBS colonization.

The sensitivity of the FA test at various incubation times was defined as the proportion of patients with positive vaginal cultures who had positive FA examinations. Specificity was defined as the proportion of patients with negative vaginal cultures who had negative FA examinations. The predictive value of a positive test was defined as the proportion of patients with positive FA examinations who had positive vaginal cultures. The predictive value of a negative test was defined as the proportion of patients with negative FA examinations who had negative vaginal cultures. Agreement was defined as the proportion of all FA examinations that agreed with culture findings.

Vertical transmission. We hypothesized that identification of GBS vaginal colonization by FA after brief ($\leq$6 h) incubation would indicate a relatively high bacterial inoculum and thus would correlate with an increased risk of colonization of newborn infants. Accordingly, we cultured throat, umbilical, rectal, external auditory canal, and gastric aspirate specimens collected at birth from two groups of infants: 35 infants (including one pair of twins) whose colonized mothers were positive by FA examination within 6 h, and 27 infants whose colonized mothers were negative by FA examination within this time interval. Four infants in the first group and two infants in the second were excluded from analysis because of intrapartum administration of ampicillin.

Infants born within the period of study who developed EOS were identified. The timing and results of FA tests performed during labor were assessed.

RESULTS

The pooled type-specific antiserum gave 4+ fluorescence with all five serotypes of GBS. Five clinical isolates each of *S. pneumoniae*, group A streptococci, and group D streptococci all fluoresced $\leq$1+. In studies of growth kinetics of small GBS inocula, growth of strain III-Bell in SBM was similar to growth in Todd-Hewitt broth with or without defibrinated sheep blood and antibiotics. GBS were reliably detectable by FA examination at concentrations of $\geq 10^5$ colony-forming units per ml, which were achieved after 6 h of incubation in SBM.

In positive clinical specimens examined by the FA technique, GBS were typically seen after brief (3 to 6 h) incubation as isolated pairs and short chains with brilliant (3+ to 4+) fluorescence. Exfoliated squamous epithelial cells were readily identifiable in most slides of properly collected specimens. GBS usually were observed to be free in the medium rather than cell associated. After overnight incubation, positive clinical specimens typically showed confluence of brightly to brilliantly fluorescing (2+ to 4+) organisms in pairs and chains.

In a pilot study of the FA technique in prenatal clinic patients, 21 of 87 vaginal swabs and 23 of 87 rectal swabs were culture positive for GBS (Table 1). At 3 h of incubation, 6 of 21 culture-positive vaginal swabs and 10 of 23 culture-positive rectal swabs (overall sensitivity, 36%) were identified by the FA technique. At 24 h of incubation, 17 of 21 culture-positive vaginal swabs and all of the culture-positive rectal swabs (overall sensitivity, 91%) were identified by the FA technique. A total of 32 false-negative results occurred. Twenty-eight of these observations occurred at 3 h of incubation and were ascribed to insufficient incubation time of a probable low initial inoculum. Of the four false-negative results obtained at 24 h of incubation, three were obvious positives on reexamination and were ascribed to fixation accidents. The other isolate was positive for GBS on the 3-h subculture but negative on subculturing at 24 h. Of the 18 false-positive results, fluorescent isolates were detected in nine cultures on reexamination. None of the isolates fluoresced brilliantly (all were $\leq$2+), but when present in small numbers, and particularly if cell associated, their paired coccal morphology and borderline fluorescence were subject to misinterpretation. These strains included three *S. aureus*, three group D streptococci, two alpha-streptococci (non-group A, B, C, D, or G), and one group A streptococcus. For nine false-positive results, none of the recultured isolates fluoresced. In subsequent work based on these observations, we required the presence of exfoliated vaginal epithelial cells, non-cell-associated organisms that fluoresced $\geq$3 to 4+, and clear-cut streptococcal morphology as criteria for a positive FA result.

Prospective evaluation of the FA test was carried out with vaginal swabs from 924 high-risk parturient women, 154 of whom were culture positive. FA results, at various time intervals of preincubation before examination, are summarized in Table 2. As in previous instances, the

TABLE 1. *GBS detection (at indicated times of incubation before FA examination) by the FA technique and culturing in vaginal and rectal swabs of prenatal clinic patients*

Incubation (h)	No. of specimens examined[a]	FA/culture result[b]				FA/culture correlation (%)				
		+/+	−/+	+/−	−/−	Sensitivity	Specificity	Predictive value (+)	Predictive value (−)	Agreement
3	174	16	28	10	120	36	92	62	81	78
24	174	40	4	8	122	91	94	83	97	93

[a] Results for vaginal and rectal swabs are combined.
[b] Number of specimens with indicated FA and culture results, respectively. GBS isolated from either subculture were considered proof of colonization.

TABLE 2. *GBS detection (at indicated times of incubation before FA examination) by the FA technique and culturing in vaginal swabs of high-risk parturient women studied prospectively on admission*

Incubation (h)	No. of specimens examined	FA/culture result[a]				FA/culture correlation (%)				
		+/+	−/+	+/−	−/−	Sensitivity	Specificity	Predictive value (+)	Predictive value (−)	Agreement
3–6	472	34	35	4	399	49	99	89	92	92
7–12	187	25	6	1	155	81	99	96	96	96
13–18	128	20	4	2	102	83	98	91	96	95
>18	924[b]	143	11	9	761	93	99	94	99	98

[a] No. of specimens with indicated FA and culture results, respectively. GBS isolated from either subculture were considered proof of colonization.
[b] Includes 137 specimens examined for the first time and 787 specimens examined a second time.

sensitivity of the test increased with increasing time intervals of incubation. Sensitivity was 49% at 3 to 6 h, exceeded 80% for isolates incubated more than 6 h, and reached 93% for isolates incubated more than 18 h. Specificity was at least 98% for isolates observed at each time interval, a reflection of our more stringent criteria for a positive FA result. The predictive values of positive and negative FA results were at least 89% at each time interval. Agreement was at least 92% at each time interval.

Since detection of GBS in vaginal swabs after brief incubation appeared to be a semiquantitative measure of colonization density, we compared rates of vertical transmission for babies whose colonized mothers were positive by the FA technique within 3 to 6 h with rates for babies whose colonized mothers were negative by the FA technique within this time interval. Of the 31 infants whose colonized mothers were rapidly identified by the FA technique, 19 (61%) were colonized with GBS at birth. Of the 31, 5 (16%) had colonization at three or more sites. Of the 25 infants whose colonized mothers were not rapidly identified by the FA technique, only 8 (32%; $P = 0.027$, determined by one-tailed Fisher exact test) were colonized at birth. Of the 25, 4 were colonized with GBS at three or more sites (16%; not significant). Colonization at multiple sites was more strongly associated with prolonged (≥12 h) rupture of membranes at delivery (eight of the nine heavily colonized patients) than with detection by the FA technique of maternal colonization at 3 to 6 h after admission.

Of the infants born to the 924 parturient women studied prospectively, four developed proven EOS due to GBS. One live-born infant with sepsis and meningitis failed to respond to resuscitative efforts at birth and died. The other three also had respiratory distress at birth but were successfully treated. The mother of the infant who died had been negative by the FA technique and vaginal culturing during an admission for premature labor 3 weeks before delivery. She was discharged undelivered and then returned with overt amnionitis at 28 weeks of gestation; she delivered soon after admission. A postpartum vaginal culture was positive for GBS. The other three mothers of infected infants all had premature followed by prolonged rupture of membranes; two had premature labor as well. The vaginal cultures from each of these women were positive. However, because of the timing of admission in each of the four cases, FA

results were not available until after delivery. Although the results were positive in each case, they did not influence either obstetrical or pediatric management decisions.

DISCUSSION

Examination of clinical specimens with pooled type-specific FA against GBS has a sensitivity approaching that of broth enrichment culture techniques after 6 h of incubation. As a technique for rapid (within 6 h) identification of GBS colonization, the technique showed only moderate sensitivity. It is possible that an early-positive FA result may serve to identify parturient women with relatively dense vaginal colonization who are at an increased risk for vertical transmission. However, since we did not identify the colonized mothers of four infants with EOS until after delivery, one can question whether the FA technique provides a practical means of identifying colonized parturient women for selective antibiotic prophylaxis.

In previous work with group-specific FA as a rapid means of identifying group A streptococci in throat swabs, Moody et al. (22) found a sensitivity of 14% for identification with direct smears and 75% after 2 h of incubation in broth. However, in studies of the use of the FA technique in pediatric office practice, Breese (11) found that the FA technique had no advantage over routine throat cultures and that its occasional use for rapid diagnosis of streptococcal pharyngitis did not justify the investment in equipment and personnel time. In Moody's studies, *S. aureus* was the most frequent cause of false-positive results, presumably because of binding of the F_c portion of fluoresceinated antibody by staphylococcal protein A (30). Control slides stained with fluoresceinated nonimmune rabbit sera and a rigid definition of streptococcal morphology (several observed chains containing at least three to five cocci) were necessary to exclude this source of error. Based on our pilot study, we adapted the same criterion for positive results but did not routinely examine control slides.

Romero and Wilkinson (26), in their original description of the use of type-specific FA for identification of GBS, reported the results of a small comparative study of 99 vaginal swabs submitted to the Bureau of Laboratories, Centers for Disease Control, Atlanta. Details of collection methods and incubation times were not given. Agreement between FA and culture precipitin results was obtained in 96 paired observations (97%). However, the authors pointed out the need for data obtained in a clinical setting to demonstrate the efficiency of FA examination as a rapid diagnostic technique.

Although other techniques for rapid identification of GBS colonization have been studied, there are relatively few data available regarding their clinical application. Fenton and Harper (13) used counterimmunoelectrophoresis with group-specific commercial antiserum for 527 clinical specimens. Of 56 cultures that showed turbidity in selective broth cultures at 6 h after collection, none of the 14 that eventually grew GBS was positive by counterimmunoelectrophoresis. At 20 h after collection, 48 of 75 cultures (64%) containing GBS were correctly identified by counterimmunoelectrophoresis. Leland et al. (19) used group-specific coagglutination with antibody-coated staphylococci as a means of identifying GBS in vaginal swabs from 80 women, using isolation of GBS from subcultured Todd-Hewitt broth as the standard of comparison. Of 14 positive swabs, 5 (46%) were identified by 6 h after inoculation, 11 (79%) by 8 h after inoculation, and 13 (93%) by 24 h after inoculation. One false-positive result was obtained. We have used coagglutination for rapid diagnosis in our own laboratory and have observed similar sensitivity but higher rates of false-positive results. Our experience with the type-specific FA technique indicates that its sensitivity and specificity are at least equal to group-specific coagglutination and clearly superior to group-specific counterimmunoelectrophoresis as a means of rapidly identifying GBS colonization.

Ancona et al. (1) and Anthony et al. (3) have demonstrated that mothers with relatively high vaginal colonization densities have significantly higher rates of vertical transmission to their newborn infants than mothers with lower bacterial counts. Furthermore, Pass et al. (24) have shown that newborn infants with three or four sites of GBS colonization at birth have significantly higher attack rates of EOS (8%) than infants with one or two sites of colonization (0.5%). In our studies of vertical transmission, the FA technique successfully identified a subgroup of colonized women whose infants were more likely to show GBS colonization at birth. However, the technique did not discriminate colonized women whose infants were more likely to be colonized at three or more sites.

The most critical issue raised by our results is one of logistics. It is primarily because of this problem that we were unable to identify before delivery the colonized mothers of four infants with EOS. Without obstetrical intervention, the mean duration of the first stage of labor ranges from 8 h in multigravidas to 13 h in primigravidas (12). On the other hand, active obstetrical management by oxytocin augmentation and ce-

sarean birth is decreasing the proportion of women whose labor exceeds 24 h (8, 10, 23). Thus, successful identification of GBS colonization by the FA technique before delivery is difficult to achieve in more than a fraction of women admitted in labor. On a 50-h-per-week schedule, we examined vaginal swabs from only 472 of 924 high-risk parturient women within 6 h of admission. Thus, our reported 49% sensitivity for the FA technique at 3 to 6 h of incubation must be tempered by the fact that only 51% of parturient women were studied within that time interval. Hence, the operational sensitivity of the procedure in our hands was only about 25%. An improvement on this performance would require round-the-clock FA examination of vaginal swabs obtained on admission, a laboratory commitment which we feel would be difficult to justify in most institutions. Other approaches to identifying colonized high-risk parturient women are clearly needed. We believe prenatal culturing of the vagina and rectum by conventional means may be a practical alternative to the use of rapid diagnostic bacteriology during labor.

ACKNOWLEDGMENTS

These studies were supported by grants HD-09700 and HD-11576 from the National Institute of Child Health and Human Development.

We gratefully acknowledge the assistance of the nursing and resident physician staff in the Labor and Delivery Suite of Michael Reese Hospital and Medical Center.

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